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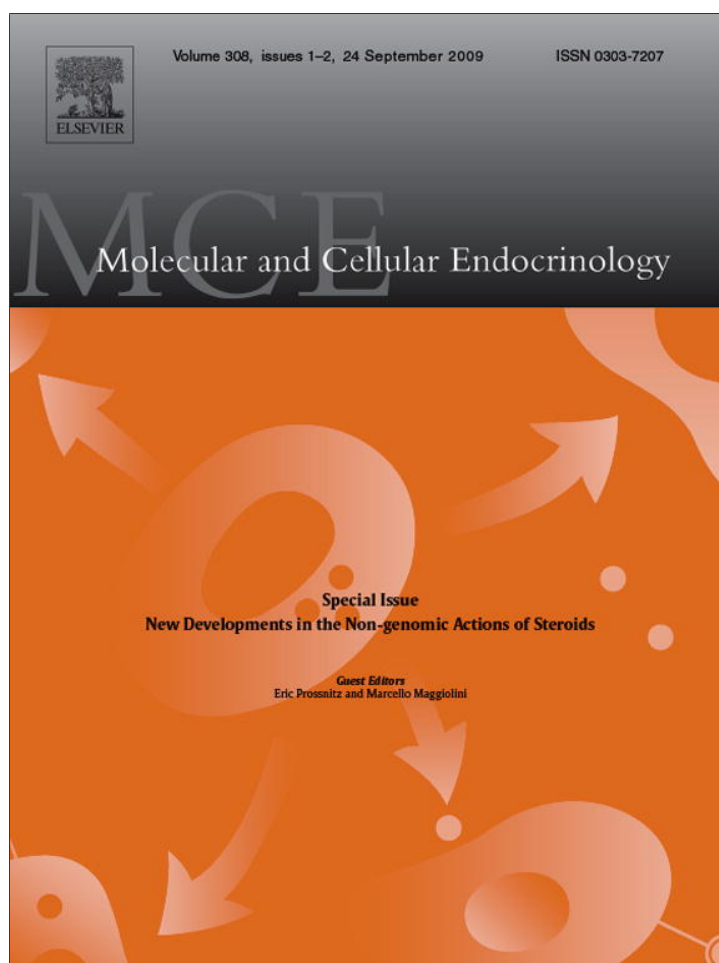
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## Review

## Nongenomic activation of spermatozoa by steroid hormones: Facts and fictions

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## ABSTRACT

The rapid effects of steroids on spermatozoa have been demonstrated for the first time two decades ago. Progesterone (P), which is present throughout the female genital tract with peaks of levels in the cumulus matrix surrounding the oocyte, stimulates several sperm functions, including hyperactivation and acrosome reaction. These effects are mediated by an extranuclear pathway, as P stimulates an influx of calcium, the tyrosine phosphorylation of sperm proteins and other signalling cascades in a rapid manner. Whether these effects are receptor mediated and which receptors mediate these effects are still a matter of discussion despite all the efforts of the scientific community aimed at identifying them during the last 20 years. Although responsiveness to P is related to sperm fertilizing ability, the physiological role of P during the process of fertilization is discussed, and recent evidence points for a role of the steroid as a chemotactic agent for sperm. A similar situation applies for estrogens (E), which have been shown to induce direct effects on sperm by an extranuclear pathway. In particular, E appear to decrease acrosome reaction in response to P, exerting a role in ensuring an appropriate timing for sperm exocytosis during the process of fertilization.

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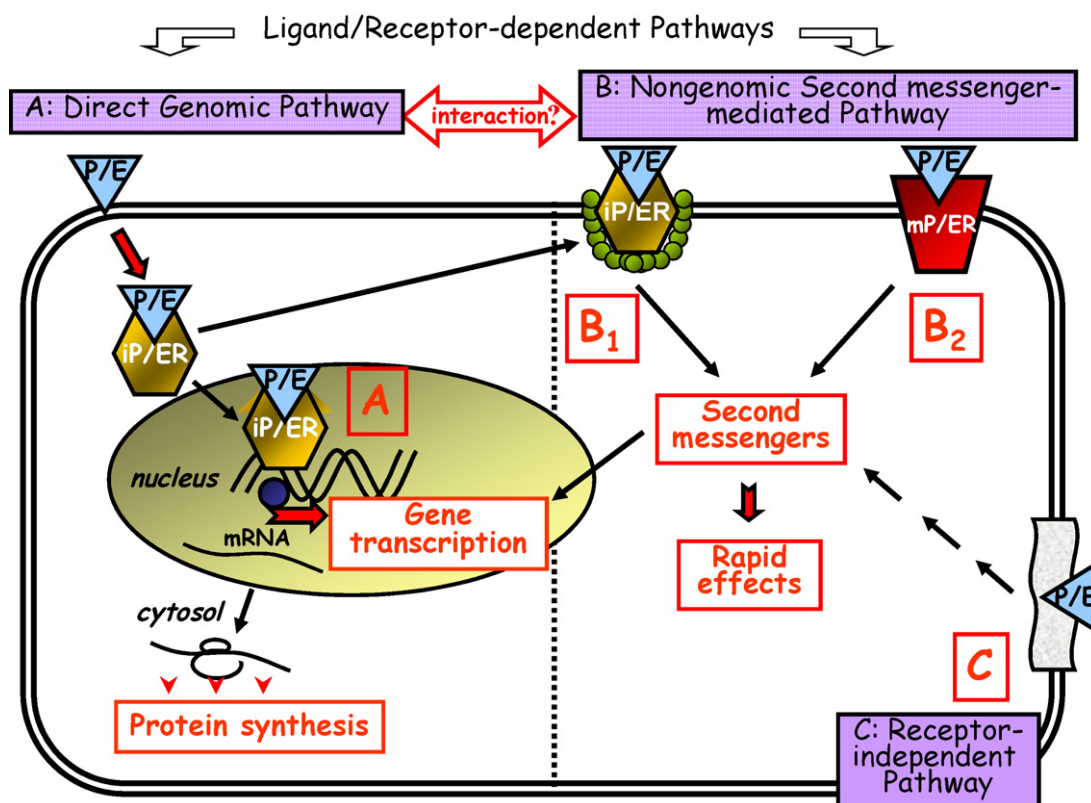
## 1. Introduction

The first demonstration of an effect of steroid hormones on spermatozoa dates back 20 years, when Calzada et al. (1988) demonstrated an increase of the membrane potential upon treatment with testosterone (T), estrogens (E) and progesterone (P).

One year later, Osman et al. (1989) reported the rapid induction of acrosome reaction (AR) by P and 17 $\alpha$ -P in human spermatozoa. At that time the occurrence of rapid, later on improperly named "nongenomic" or "membrane-initiated", effects of steroids, had been reported for neurosteroids, in particular for 5 $\alpha$ -reduced, 3 $\alpha$ -hydroxylated pregnane derivatives (Mendelson et al., 1987), and for vitamin D (Nemere and Norman, 1987). The concept that steroid hormones may have effects besides the classic (genomic) ones has not been immediately accepted by the scientific community (the endocrinologists, in particular) and the demonstration that steroids may induce effects in genomically inert cells like spermatozoa has probably contributed to strengthen the idea that such

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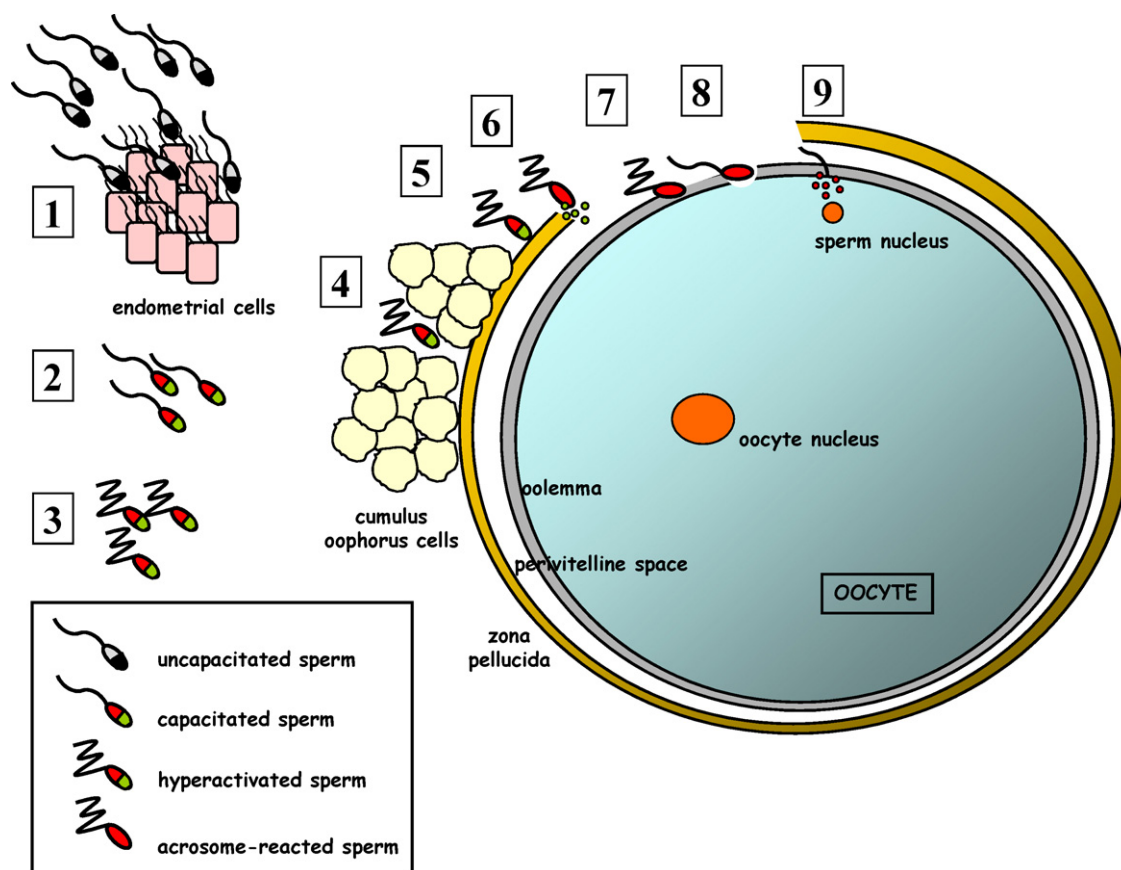


**Fig. 1.** Cross-talk between the different signalling pathways and PR/ER types involved in mediating the P/E effects in the cell. (A) Direct genomic pathway through classic cytosolic/nuclear receptors (iP/ER) acting on gene transcription. (B) Nongenomic second messenger-mediated pathways leading to rapid effects through classic cytosolic/nuclear receptor spanning in the plasma membrane associated in the caveolae (B1) or through unusual membrane receptor (mP/ER, B2). Both pathways are P/E-dependent and receptor-mediated. (C) Receptor-independent pathway involving an P/E-induced perturbation of the plasma membrane. : mRNA synthesizing ribosome : classic intracellular P/ER : membrane perturbation : novel membrane P/ER : caveolae.

effects can occur. In addition, there is an universal agreement that the effects of P on sperm are observed also when non-permeable P conjugates are used and are not counteracted by classical P antagonists such as mifepristone (also known as RU487), strengthening the idea that these effects are “extranuclear” and “nongenomic” in nature (Correia et al., 2007). The first report on the effects of P on sperm has been followed by publication of many papers (up to now almost 300 can be found in Medline using P, sperm and AR as keywords), attempting to define the molecular mechanisms underlying these actions as well as to identify the “putative” receptors involved and their role in the process of fertilization. Now we know which molecular pathways are activated by P in spermatozoa (Fig. 1), but, despite many efforts, the identity of the sperm membrane receptor for the steroid remains elusive (see paragraph 4). Similarly, despite the demonstration that responsiveness to P is related to fertilization, whether the steroid is a physiological activator of sperm is still discussed by some scientists (see paragraph 3). Lately, the potential effect of estrogens and endocrine disruptors (EDs) on claimed decline of male fertility has been investigated. Even if the possible targets of actions of E and ED are spermatogenesis, the ability of E to interact with P in modulating acrosome reaction was revealed as well (see paragraph 3). The present review summarizes and analyzes critically the main discoveries in the last years on the molecular pathways that are activated by P and E in sperm, the pathophysiological relevance of the effects of these hormones and the possible involvement of receptors. Owing to the important differences in the reproductive process and male gamete biology among mammalian species this review is focusing with results obtained in human sperm unless otherwise indicated.

## 2. Molecular pathways and functions activated by progesterone and estrogens in sperm

In the last two decades there have been reports demonstrating that P may *in vitro* stimulate several functions in human sperm (namely: hyperactivated motility, capacitation, chemotaxis and acrosome reaction). All these functions are involved in the process of fertilization of the oocyte (Fig. 2) and occur physiologically in the female genital tract. Indeed, immediately after ejaculation spermatozoa are unable to fertilize, a quality that they acquire during transit in the female genital tract following interaction with local factors (reviewed in Muratori et al., 2009). In order to become able to fertilize, spermatozoa must undergo a complex event known as capacitation at the end of which sperm acquire hyperactivated motility (a special type of progressive motility, characterized by disordered movement of the head and tail needed to penetrate oocyte vestments) as well as the ability to respond to stimuli leading to acrosome reaction, an exocytotic process which helps the sperm to penetrate the zona pellucida surrounding the oocyte. Chemotaxis is believed to occur in the proximity of the oocyte and is involved in directing the sperm in the last part of its journey. P has been shown to activate several signalling pathways (generation of cAMP, increase of intracellular calcium ( $\text{Ca}^{2+}$ ), promotion of tyrosine phosphorylation of proteins, activation of phospholipases and many others), all of which have been demonstrated as being involved in the regulation of these functions. However, at present, the picture of the signalling pathways that are activated and, most importantly, which of them are required for induction of a particular sperm function, are not clear.



**Fig. 2.** Sperm activation phases required for fertilization. During their transit through the female genital tract spermatozoa undergo a sequence of activating events which render them able to fertilize. (1) Sperm interaction with endometrial cells; (2) capacitation; (3) hyperactivation; (4) penetration through the cumulus oophorus cells; (5) sperm binding to the zona pellucida; (6) acrosome reaction; (7) sperm binding to oolemma; (8) fusion between sperm and oocyte plasma membranes; (9) injection of sperm nucleus into the oocyte.

The situation concerning direct effects of E (including environmental E) on spermatozoa is even more intricate. Indeed, although E have been shown to activate some signalling pathways in spermatozoa, whether they are associated with one or more sperm functions is discussed.

### 2.1. P-induced sperm capacitation

As mentioned above, capacitation is a complex event occurring in sperm following their release in the female genital tract that ultimately prepares and allows sperm to reach the oocyte and to become reactive to stimuli that induce AR *in vivo*. *In vitro*, the process is achieved in the presence of both bicarbonate and albumin, inducing respectively soluble adenylate cyclase activation and generation of cAMP, and an increase of membrane fluidity by promoting the efflux of cholesterol from the plasma membrane. There are reports suggesting a positive effect of P on capacitation of human sperm (Kay et al., 1994; de Lamirande et al., 1998; Thundathil et al., 2002; Yamano et al., 2004). This effect is, however, obtained with high concentrations (1–30  $\mu$ M) of the steroid and its physiological relevance is doubtful (discussed in the next paragraph). In addition, there is no universally recognized marker or endpoint to define capacitation, making comparison between these studies very difficult, due to the fact that different markers have been used to define it. Induction of capacitation by P appears to be related promotion of generation of oxygen species by sperm (de Lamirande et al., 1998), activation of the mitogen-activated protein kinase (MAPK) but not of the cAMP/PKA cascade (Thundathil et al., 2002). Indeed, short-term application of micromolar concentrations of P activated MAPK

in sperm (Luconi et al., 1998c), but, interestingly, this effect was not related to AR induction (Luconi et al., 1998c). Since a previous work from our laboratory demonstrated an involvement of MAPK in sperm capacitation (Luconi et al., 1998a), these studies seem to indicate that the *in vitro* effect of P on capacitation, if any, is mediated by this pathway.

### 2.2. P-induced sperm motility

Forward motility represents the necessary requisite for sperm to reach the oocyte. In the proximity of the latter, spermatozoa acquire a particular type of motility (namely hyperactivated motility) characterized by disordered movements of the head and flagellum (Luconi et al., 2006). Many factors affect sperm motility, including calcium, cAMP, and constituents found in the female genital tract (Luconi et al., 2006). A positive effect of P (0.1–1  $\mu$ M) on forward (Contreras and Llanos, 2001) and hyperactivated (Uhler et al., 1992; Calogero et al., 1996; Jaiswal et al., 1999) motility has been reported by some authors but not confirmed by others (Kay et al., 1994; Wang et al., 2001; Luconi et al., 2004). The effect of P on hyperactivated motility was found to be transient and was not counteracted by P antagonists (Uhler et al., 1992; Yang et al., 1994). Recently, it has been found that P induces  $\text{Ca}^{2+}$  oscillations, due to a release from intracellular stores (see also next) in the proximity of the flagellum, which are strictly related to the flagellar activity (Harper et al., 2004; Bedu-Addo et al., 2007), suggesting that this mechanism, rather than the massive  $\text{Ca}^{2+}$  entry stimulated by the steroid, is responsible for induction of hyperactivation. Such effects were observed at micromolar concentrations of P, in the presence of EGTA in the



external medium, and, as mentioned above, were associated with changes in the flagellar beating, a typical feature of hyperactivated motility that occurs in the proximity of the oocyte (Bedu-Addo et al., 2007). The fact that only a small fraction of spermatozoa shows  $\text{Ca}^{2+}$  oscillations may provide an explanation for the conflicting results concerning the effects of the steroid on sperm motility reported in the previous papers.

It has been reported that P, at micromolar concentrations, stimulates an increase of cAMP intracellular levels in sperm (Parinaud and Milhet, 1996), however, whether activation of soluble adenylylase and thus cAMP generation are required for hyperactivated motility in human sperm is not clear (Marquez and Suarez, 2008) and the involvement of this system in the increase of motility stimulated by the steroid requires further studies.

### 2.3. P-induced sperm chemotaxis

Sperm chemotaxis plays an essential role in species with external fertilization (Friedrich and Jülicher, 2007) and has been demonstrated to occur also in mammals (Sun et al., 2005). The process takes place in the proximity of the fertilization site and it is initiated by the release of factors from the oocyte and the surrounding cumulus cells. Whether P or other steroids present in the follicular fluid or in the cumulus matrix may be responsible for this chemoattractant effect is still an open question. As for the effects on hyperactivated motility, conflicting results have been reported also on the effects of P on sperm chemotaxis (Villanueva-Díaz et al., 1995; Jaiswal et al., 1999; Wang et al., 2001; Teves et al., 2006). It must be remarked that evaluating sperm chemotaxis is very difficult and the use of videomicroscopy and computer image analysis appears to be the only most reliable and direct way to investigate directionality of sperm toward a chemoattractant. Using this approach, Teves et al. (2006) recently showed that P is chemoattractant for human spermatozoa at nanomolar concentrations. Of interest, application of a P gradient (starting from 0.3 nM) induces slow  $\text{Ca}^{2+}$  oscillations in the caudal part of the head of about 30% of sperm (Harper et al., 2004).  $\text{Ca}^{2+}$  oscillations in response to P do not appear to be dose-dependent and as mentioned above, occur in the absence of calcium in the external medium and seem to be involved in the modulation of flagellar activity (Bedu-Addo et al., 2007) producing a type of flagellar beating which is required for a chemotactic response. However, a direct relationship between modification of motility parameters and chemotaxis is lacking and further studies are needed to establish it.

### 2.4. P-induced acrosome reaction

AR occurs *in vivo* following sperm interaction with the oocyte zona pellucida (Hoodbhoy and Dean, 2004) and consists of fusion and fenestration of external and acrosomal membrane of sperm allowing the release of acrosomal content (mostly proteolytic enzymes). AR can be induced by many constituents present in the female genital tract. Among these, P has been intensively studied in the last decade, in view of its high concentrations in the genital tract fluids and in the oocyte cumulus mass, where the steroid reaches micromolar concentrations. In view of the role of  $\text{Ca}^{2+}$  in induction of AR, there are no doubts that the massive influx of the ion stimulated by P can trigger the process (Correia et al., 2007; Muratori et al., 2009). The wave of intracellular  $\text{Ca}^{2+}$  stimulated by P in sperm is biphasic, composed of a transient increase followed by a sustained elevation (Blackmore et al., 1991; Baldi et al., 1991) and is obtained at concentrations ranging from 10 nM to 100  $\mu\text{M}$  (Luconi et al., 1998b). The response is totally dependent on extracellular  $\text{Ca}^{2+}$  and is clearly dose-dependent (Luconi et al., 1998b; Harper et al., 2003). Investigating the involvement of membrane  $\text{Ca}^{2+}$  channels has been the aim of many studies, but

at present, which channels (voltage-operated? receptor-operated?) are involved in responsiveness to P in human sperm remains elusive (Bonaccorsi et al., 2001; Guzmán-Grenfell and González-Martínez, 2004). Recently, evidence has been reported for mobilization of  $\text{Ca}^{2+}$  from sperm intracellular stores in response to P (Bedu-Addo et al., 2007), indicating that multiple mechanisms are involved in the response. Indeed, in the absence of extracellular  $\text{Ca}^{2+}$  (EGTA-buffered medium), a small percentage of cells (about 5%) shows a tiny  $\text{Ca}^{2+}$  transient, suggesting mobilization from intracellular stores. The nature of such intracellular stores in spermatozoa is still discussed (Bedu-Addo et al., 2008).

Of interest, whereas the  $\text{Ca}^{2+}$  response occurs in the vast majority of P-stimulated sperm, as demonstrated by image analysis of fura2-loaded spermatozoa (Kirkman-Brown et al., 2000), AR in response to the steroid occurs only in a minor percentage (10–15) of them (Baldi et al., 1991). Such a difference is too high to reflect the diverse sensitivity of the methods used to detect sperm intracellular  $\text{Ca}^{2+}$  and AR and appears to underlie the necessity for the simultaneous activation of other pathways for the attainment of sperm exocytosis. On the other hand, other pathways have been demonstrated as being activated by P, but their involvement in the induction of AR is still controversial. For instance, although some studies demonstrate an involvement of the cAMP/PKA pathway in the induction of AR in sperm (Harrison et al., 2000), others do not (Moseley et al., 2005). Indeed, the increase in cAMP concentrations following P stimulation is very low (Parinaud and Milhet, 1996), raising doubts about the relevance of the pathway in the response to the steroid. A similar situation applies for the phospholipase C/inositol3phosphate/diacylglycerol/protein kinase (PLC/IP3/DAG/PKC) pathway: although the enhancing effect of P on inositol trisphosphate (Thomas and Meizel, 1989) and diacylglycerol (O'Toole et al., 1996a) synthesis has been reported, blockers of phospholipase C do not appear to affect P-stimulated  $\text{Ca}^{2+}$  fluxes (Harper et al., 2004), and whether the pathway is involved in the induction of AR remains controversial (O'Toole et al., 1996b; Bonaccorsi et al., 1998). P at micromolar concentrations has been also shown to promote tyrosine phosphorylation of sperm proteins (Tesarik et al., 1993; Luconi et al., 1995) and this pathway is involved in AR and  $\text{Ca}^{2+}$  influx in response to the steroid (Martínez et al., 1999; Bonaccorsi et al., 1995). However, as for cAMP generation, tyrosine phosphorylation increase in response to P is small (Luconi et al., 1995; Kirkman-Brown et al., 2002) and its influence on  $\text{Ca}^{2+}$  transients and AR is discussed (Kirkman-Brown et al., 2002). Recently, we have demonstrated that the tyrosine kinase Src is involved in P-induced  $\text{Ca}^{2+}$  influx and AR in human sperm (Varano et al., 2008). In particular, we have shown that inhibition of Src activation decreases  $\text{Ca}^{2+}$  influx in response to the steroid, affecting both the peak and the plateau phases of the response. As a consequence, AR in response to P is suppressed. These data suggest that Src may be stimulated by P upstream of  $\text{Ca}^{2+}$  increase, in line with other results of our and other groups demonstrating that the plateau phase of P  $\text{Ca}^{2+}$  response is inhibited by generic inhibitors of tyrosine kinases in spermatozoa (Bonaccorsi et al., 1995; Tesarik et al., 1996). Src, which is located in the sperm post-acrosomal region, may be involved in the release of  $\text{Ca}^{2+}$  from sperm intracellular stores (such as the redundant nuclear envelope; Ho and Suarez, 2003), which are located in the same area. At present, the mechanism leading to Src activation by P in sperm is not known.

### 2.5. Effects of estrogens and xenoestrogens on sperm

That E are important for male reproductive functions became clear following the development of E receptor knock out (ERKO) mice, which show important alterations of spermatogenesis and infertility (Eddy et al., 1996) due to modifications in the expression of a battery of genes involved in spermatogenesis and fluid re-

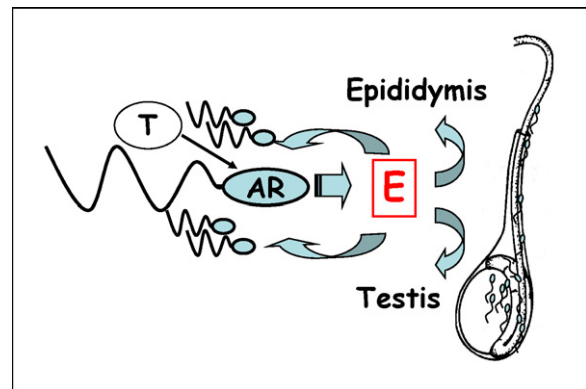
sorption in the reproductive tract (Zhou et al., 2001). In addition, it has been recently shown that a polymorphism in the promoter of the ER $\alpha$  is related to a lower sperm count in the ejaculate (Guarducci et al., 2006). Besides the genomic effects, evidence has emerged concerning direct, extranuclear, effects of E (Fig. 1) on mouse spermatogenic cells, where the steroid rapidly stimulates a transient increase in ERK activation (Vicini et al., 2006). Studies concerning extranuclear effects of E in mature spermatozoa are conflicting. Our group (Luconi et al., 1999) has shown that a short time *in vitro* treatment of human sperm with E induces a small rise of intracellular Ca<sup>2+</sup> which interferes with the subsequent Ca<sup>2+</sup> wave stimulated by P. Ultimately, this effect determines a reduction in P-induced AR, although E themselves do not appear to affect the process. These results have been recently confirmed by another group (Vigil et al., 2008). Similar results were obtained with the ER antagonists tamoxifen and ICI 164384 (Luconi et al., 2001), suggesting that the putative receptor involved in the estrogenic response in spermatozoa shows different characteristics respect to classical ERs (see Section 4). Conversely, no effects on spermatozoa were observed with two xenoestrogens, namely bisphenol A and octylphenol polyethoxilate (Luconi et al., 2001). In a subsequent study, Francavilla et al. (2003) reported that pre-incubation with E does not alter the ability of human sperm to fuse with the oocyte nor interferes with the enhancing effect of P on the process. Conversely, Adeoya-Osiguwa et al. (2003) reported a stimulatory effect of E and different xenoestrogens on both capacitation, acrosome reaction and fertilizing ability of mouse spermatozoa. Using mycotoxins with E-like action, Tsakmakidis et al. (2006) recently reported an inhibitory effect on spontaneous boar sperm AR. Finally, Aquila et al. (2004) hypothesize a role for E in human sperm survival by stimulation of the PI3K/Akt and ERK pathways. Interestingly, at difference with the other studies, this study reported inhibition of the effects by classical ER antagonists.

### 3. Pathophysiological relevance of the steroid effects on sperm

As many of the maturation processes and functional events that characterize sperm life after release from the testis occur in the female genital tract, there is no doubt that factors present in this environment, and steroid hormones in particular, have a potential role in their modulation. Capacitation, development of hyperactivated motility, chemotaxis and AR are all events occurring in the female reproductive tract and potentially modulated in this contest.

As capacitation is needed to prepare sperm to undergo AR, it is believed to occur in the uterus or in the fallopian tube just before spermatozoa reach the oocyte. In this situation, capacitation should be induced by P at the concentration found in the tubal fluid, which is lower than the one found in the cumulus matrix (Munuce et al., 2006). Conversely, the effects of the steroid on capacitation are observed at high concentrations of the steroid, similar to those found in the cumulus matrix, raising doubts about their physiological significance in the process.

At a first glance, induction of AR by P (which is present throughout the female genital tract, with its maximal levels in the cumulus matrix) may be surprising. An acrosome-reacted sperm does not retain the ability to fertilize even if AR occurs in the proximity of the oocyte, and thus premature AR should be avoided. However, at the concentrations found in the cumulus mass, P induces AR and a fertilizing sperm needs to pass through the cumulus to fertilize an egg. In this light, the inhibiting effect of E on P-induced AR (Luconi et al., 1999; Vigil et al., 2008) may have a physiological relevance: the interplay between the two steroids during sperm transit in the cumulus mass may be important to ensure a correct timing of the process. In addition, the effects of P present in the cumulus matrix



**Fig. 3.** The sperm as a “mobile endocrine unit”. Spermatozoa can convert testosterone (T) into estrogens (E) during their passage from the testis to the epididymis through the expression of an endogenous aromatase (AR), thus acting both in a paracrine and autocrine loop on the structures and cells surrounding.

may be involved in priming the sperm to the subsequent effects of the zona pellucida proteins (Roldan et al., 1994). Finally, a physiological effect of P in removing poor quality spermatozoa by inducing premature AR (Harper and Publicover, 2005) cannot be excluded. It is important to mention that P-induced AR is related to the sperm fertilizing ability (Krausz et al., 1996; Jacob et al., 1998), and that responsiveness to P is decreased in subfertile patients (Falsetti et al., 1993), strongly suggesting that the ability of a spermatozoon to respond to the steroid has to be considered a pre-requisite for fertilization.

If the physiological relevance of induction of capacitation and AR by P is a matter of discussion, the effects on motility and chemotaxis could be considered the primary physiological functions of the steroid. As mentioned above, there are reports showing that the effects of P on the three processes occur *in vitro* at concentrations of the steroid similar to those found in the areas of the female genital tract where they occur. In addition, P-stimulated hyperactivated motility and chemotaxis appear to occur in a small fraction of spermatozoa (Harper et al., 2004; Teves et al., 2006), suggesting that a sperm selection is needed and takes place during the journey to the oocyte. In this light, it is possible that P may induce hyperactivation only in those sperm that possess all the requisites to fertilize the oocyte.

A part from the mentioned effect in limiting P-induced AR, the rapid effects of E in spermatozoa do not appear to have a direct role on sperm-oocyte interaction (Francavilla et al., 2003). However, it is worth mentioning that aromatase mRNA have been found in sperm and their levels are lower in the immotile sperm fraction (Carreau et al., 2008), suggesting a possible role of E produced by sperm from androgens present in the male genital tract in the development of motility. This effect, if any, does not appear to be mediated by a genomic pathway, as the observed actions of E are rapid and the presence of classical ERs in mature sperm is uncertain (Luconi et al., 1999; Aquila et al., 2004; Carreau et al., 2006). E derived from testosterone conversion by aromatase present in spermatozoa, may be also involved in the regulation of ER-activated pathways in the epididymis or in the testis (Fig. 3), which have the potential to regulate sperm maturation at these levels (Hess et al., 2001). Moreover, a direct autocrine as well as paracrine effect on modulation of sperm activation may be exerted by the self production of E in the sperm from T conversion (Fig. 3).

The effects of endocrine disruptors on male reproductive functions appear to be even more complex. There are reports that consider EDs responsible for the claimed global decrease in semen quality. It must be considered that there is no agreement on this point (see also Fisch, 2008), and that it is probably a localized

more than worldwide phenomenon. Decline in semen quality has been suggested to be associated to male pre-natal exposure to EDs, and *in vitro* and *in vivo* laboratory data seem to confirm such an association (Giwerzman et al., 2007), although further studies are awaited. Human beings are increasingly exposed to EDs (for instance to compounds like cadmium and bisphenol A, which have been demonstrated to bind estrogenic receptors) and there is evidence that some EDs may disrupt steroidogenesis in the Leydig cells (Takiguchi and Yoshihara, 2006; Kumar et al., 2008), which may be reflected in impaired spermatogenesis. In particular, triclosan (Kumar et al., 2008) appears to reduce testosterone production by Leydig cells by decreasing the activity of a membrane adenylate cyclase, whereas cadmium (Takiguchi and Yoshihara, 2006) affects androgens production by directly binding to androgen and estrogen receptors. EDs have been also demonstrated to rapidly disrupt the integrity of the blood-testis barrier in the seminiferous epithelium (Fiorini et al., 2004, 2008). However, it is not clear whether these effects are mediated by binding to steroid receptors and/or a non genomic mechanism. The recent demonstration that E exerts rapid effects on germ cells (Vicini et al., 2006), suggests that an interplay between the rapid and classical actions may occur during spermatogenesis, and EDs may thus interfere with the process both by classical and non genomic mechanisms.

#### 4. Are the steroid effects on sperm receptor-mediated?

The involvement of a receptor-mediated mechanism in the action of P has been proposed based on different findings, including the increase of sperm responsiveness to P following capacitation (Baldi et al., 1991) and the lack of a response to a second challenge with the steroid (Luconi et al., 1998). The ability of membrane-impermeant conjugates of P to exert similar effects as the native compound seems to indicate that this receptor is located at the membrane. However, the use of protein-conjugated steroids has been criticized because they can release the free compound in unknown amounts. Moreover, big proteins, such as BSA, can have effects on sperm *per se* (Meizel, 1985).

Several hypothesis can be made concerning the type of E and P receptor mediating the rapid effects of the steroid on spermatozoa (Fig. 1), namely: the classical P or ER located at the membrane (B1), a new receptor located at the membrane (B2) or occurrence of membrane-perturbing effects of the steroids (C).

Although the classical P receptors have not been detected in spermatozoa (Sabeur et al., 1996; Luconi et al., 1998b), an antibody directed against the C-terminal region of the P receptor, able to reduce AR and  $Ca^{2+}$  influxes induced by the steroid, recognizes protein bands of 54–60 kDa in human spermatozoa (Sabeur et al., 1996; Luconi et al., 1998; Shah et al., 2005) but so far, whether these proteins represent the sperm receptors for the steroid remains unclear. A G-protein coupled, membrane P receptor (mPR) has been cloned in different species including the human (Zhu et al., 2003), but attempts to link this receptor to the one responsible for the membrane effects of the steroid in sperm have been unsuccessful. In addition, the human homolog of the mPR has been recently demonstrated as localizing to the endoplasmic reticulum and being unresponsive to P (Krietsch et al., 2006). More recently, an extranuclear P receptor has been identified in human sperm by proteomic analysis (Baker et al., 2007), although its involvement in induction of AR or other P effects is awaited. Given the numerous candidates for the receptor, the possible involvement of a molecular complex, as recently suggested by Correia et al (2007), is attractive. For a more complete updated review on membrane progesterone receptor see Fernandez et al. (2008).

The situation concerning E is similar: as mentioned above, results about the presence of classical ERs are conflicting, and although some E binding proteins have been found in mature

spermatozoa (Luconi et al., 1999; Carreau et al., 2006), their involvement in the rapid effects of the steroid has not been unequivocally demonstrated. Finally, the possibility that a non-receptor mechanism may be involved for both steroids cannot be excluded. The high concentrations needed to obtain the effects and the observation that membrane perturbing effects occur in sperm following challenge with P or E (Shivaji and Jagannadham, 1992) represent a convincing alternative to a receptor mechanism.

#### 5. Concluding remarks

After 20 years from the discovery of rapid effects of P on spermatozoa, most of the signalling pathways involved have been disclosed, but still many questions remain to be solved. As discussed above, the main physiological role of P in sperm may be related to stimulation of hyperactivated motility and chemotaxis: however, further studies are needed to confirm these effects as well as to better clarify the signalling pathways involved in these specific effects. Concerning the effects of P on induction of AR, which occur at micromolar concentrations of the steroid, it must be considered that spermatozoa must cross the cumulus mass before reaching the zona pellucida, being here exposed to elevated concentrations of the steroid. It remains to be established which is the final effect of this situation on sperm. The time needed to cross the zone could be long enough to provoke an untimely induction of AR (possibly excluding that sperm from the process of fertilization). On the contrary, the exposition to P could simply sensitizes the sperm for the subsequent action of zona proteins (Roldan et al., 1994). In both cases, a physiological role of the effect of P cannot be excluded: indeed, those sperm that eventually undergo AR during crossing of the cumulus may be considered as kamikaze sperm facilitating the passage of the one committed to fertilize.

Although most of the current evidence suggests the involvement of a receptor mechanism in the action of P, identification of this receptor, which may give hints to studies on contraceptive vaccines and on therapeutical approaches to male infertility, is still lacking. Alternative mechanisms (for instance, effects on membrane fluidity, which cannot be excluded when stimulating a cell with such high concentrations of a steroid molecule) should be also taken into consideration.

Concerning E, there is evidence that estrogenic compounds with endocrine disrupting properties may affect spermatogenesis in animals exposed during their uterine life, however, at the moment, it is not clear the relative contribute of rapid vs classical pathways. For some of the EDs studied, evidence for direct binding to estrogen receptors is lacking. A direct effect of E on sperm appears to be important for ensuring an appropriate timing for sperm activation during the process of fertilization, however, the mechanisms and the receptor involved are still quite obscure and require further studies. The recent demonstration that G protein receptor 30 (GPR30) mediates the proliferative effects of E in spermatogonial cells in the mouse (Sirianni et al., 2008), opens new perspectives in the field prompting scientists to investigate the involvement of such receptor in the physiology of human sperm.

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